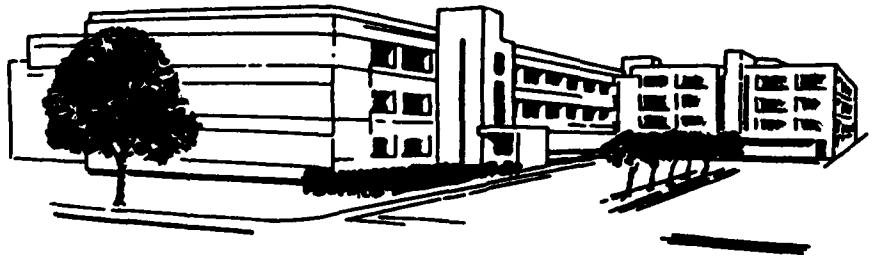
INSTITUTE REPORT NO. 457

Assessment of 7.5% NaC1 /6% Dextran-70 (HSD) Effects on Serum or Plasma Protein Determinations

M.A. Dubick,
J.J. Summary,
J.Y. Greene,
J.W. Holcroft
and C.E. Wade

Division of Military Trauma Research

December 1990



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Assessment of 7.5% NaCl /6% Dextran-70 (HSD) Effects on Serum or Plasma Protein Determinations, M.A. Dubick et al

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

The experimental studies of the author described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research. The Manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the author adhered to the "Guide for the Care and Use of Laboratory Animals," DHEW Publication (NIH) 85-23.

Human Subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Reg 50-25 on the use of volunteers in research.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

Donald R. Corby 9 Jan 97

DONALD R. CORBY (date)
COL, MC
Commanding

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release Distribution is unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No. 457	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Letterman Army Institute of Research 6b. OFFICE SYMBOL (If applicable) SGRD-ULT-M	7a. NAME OF MONITORING ORGANIZATION US Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code) Division of Military Trauma Research LAIR Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick, Frederick, MD 21701
8a. NAME OF FUNDING / SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63807A PROJECT NO. D836 TASK NO. AX WORK UNIT ACCESSION NO. 087
11. TITLE (Include Security Classification) (U) Assessment of 7.5% NaCl/6% Dextran-70 (HSD) Effects on Serum or Plasma Protein Determinations	
12. PERSONAL AUTHOR(S) M.A. Dubick, J.J Summary, J.Y. Greene, J.W. Holcroft, C.E. Wade	
13a. TYPE OF REPORT Institute 13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) 1990 December 26 15. PAGE COUNT 14
16. SUPPLEMENTARY NOTATION	
17. COSATI CODES FIELD / GROUP / SUB-GROUP	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) (U) Hypertonic Saline, Dextran-70, Plasma protein, Trauma, biuret, refractometry (U)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

For the past 25 years, reports have appeared in the literature citing dextran interference with accurate plasma or serum protein determinations, particularly if assayed by the biuret method. In our evaluations of the overall effects of HSD, we have monitored changes in serum or plasma protein concentrations following hemorrhage and subsequent resuscitation. The present study investigates protein concentrations in human, rabbit, and swine serum or plasma following a single i.v. infusion of 7.5% NaCl/6% Dextran-70 (HSD) at 4 ml/kg, or daily doses at the maximum tolerated dose of 16 ml/kg for 14 days. Protein concentrations were determined by modified Lowry, dye-binding, and an automated biuret method, as well as by refractometry, before, and at various times following HSD infusion in both euvolemic and hemorrhaged animals. Other studies analyzed plasma protein concentrations from 13 human trauma patients infused with 250 ml of HSD.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Donald G. Corby, COL, MC	22b. TELEPHONE (Include Area Code) (415) 561-3600 22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.*

In in vitro studies, dextran appeared to contribute to the protein concentrations determined by the biuret assay or refractometry when dextran serum concentrations exceeded 1.2 g/dl. The in vivo studies indicated, however, that in humans and experimental animals, infusion of HSD at proposed therapeutic levels does not interfere with plasma protein determinations, but at extremely high dextran concentrations, dye-binding assays appear more reliable.

Assessment of 7.5% NaCl/6% Dextran-70 (HSD) Effects on Serum or Plasma Protein Determinations

Michael A. Dubick[1]*, James J. Summary[1],

Jacqueline Y. Greene[1], James W. Holcroft[2]

and Charles E. Wade[1]

[1]Division of Military Trauma Research
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

[2]Department of Surgery
University of California
School of Medicine
Davis, CA 95616

Abstract

For the past 25 years, reports have appeared in the literature citing dextran interference with accurate plasma or serum protein determinations, particularly if assayed by the biuret method. In our evaluations of the overall effects of HSD, we have monitored changes in serum or plasma protein concentrations following hemorrhage and subsequent resuscitation. The present study investigates protein concentrations in human, rabbit, and swine serum or plasma following a single i.v. infusion of 7.5% NaCl/6% Dextran-70 (HSD) at 4 ml/kg, or daily doses at the maximum tolerated dose of 16 ml/kg for 14 days. Protein concentrations were determined by modified Lowry, dye-binding, and an automated biuret method, as well as by refractometry, before, and at various times following HSD infusion in both euvolemic and hemorrhaged animals. Other studies analyzed plasma protein concentrations from 13 human trauma patients infused with 250 ml of HSD. In in vitro studies, dextran appeared to contribute to the protein concentrations determined by the biuret assay or refractometry when dextran serum concentrations exceeded 1.2 g/dl. The in vivo studies indicated, however, that in humans and experimental animals, infusion of HSD at proposed therapeutic levels does not interfere with plasma protein determinations, but at extremely high dextran concentrations, dye-binding assays appear more reliable.

Assessment of 7.5% NaCl/6% Dextran-70 (HSD) Interfere with Serum or Plasma Protein Determinations -- Dubick et al.

Introduction

Dextrans and crystalloid solutions have been employed for decades for the treatment and management of hypovolemic states and hemorrhagic shock. While it has been shown that hypertonic crystalloid solutions can induce an improvement over conventional therapy in cardiovascular recovery following hemorrhage (1), more recently, combining hypertonic saline (7.5%) with 6% Dextran-70 (HSD) has been introduced as potentially improved therapy (2). This introduction of HSD has generated a number of experimental studies investigating its safety and efficacy (3-6) and HSD is currently undergoing Phase III clinical trials in the U.S.

A component of these experimental studies has evaluated changes in protein concentrations as an index of plasma volume expansion following infusion of hypertonic solutions (7) as well as the effects of Dextran-70 on protein and albumin fluxes following traumatic injury (8). In addition, in response to potential allergic reactions to dextrans, recent studies have addressed effects of HSD on plasma immunoglobulins, where accurate determination of plasma protein concentrations is an important component (9).

These studies however, are potentially compromised by continuing reports in the literature which state that dextrans interfere with serum protein determinations (10-12). Early reports noted that serum containing clinical Dextran-40, 70 and 75 became turbid when biuret reagent was added (12,13) and consequently resulted in abnormally high serum protein readings. Further investigation revealed that turbidity was the result of formation of an insoluble complex of dextran with copper and tartrate (or EDTA) in a strongly alkaline solution and turbidity depended upon the tartrate concentration (12,13). As a consequence, a number of studies have shown that modifying the biuret reagent or centrifuging the samples could eliminate this interference (13-15) and others have used the Lowry assay to avoid dextran-induced interference (8).

Nevertheless, the biuret method continues to be the most widely used protein assay in clinical laboratories. With most labs using automated technology, it is unlikely that technicians would be aware that a particular patient had received dextran. Therefore, to better evaluate the potential for dextran-induced interference of serum protein determinations, the present study compared and evaluated serum protein measurements using 4 assay techniques following infusion of HSD at doses ranging from the proposed single therapeutic dose of 4ml/kg to daily infusions of the maximum tolerated dose (MTD) of 16ml/kg in experimental animals, as well as following a single infusion of 250 ml of HSD in trauma patients.

Material and Methods

Animals and Treatment

Immature (21.6±0.6 kg) Yorkshire swine (J.G. Boswell Co., Corcoran, CA) were randomly assigned to the control or hemorrhaged group and chronically instrumented as previously detailed (5). Pigs in the hemorrhaged group were progressively bled 25 ml/kg over a 1 hr period and then pigs in both groups were infused intravenously with 4 ml/kg body weight with the 7.5% NaCl/6% Dextran-70 (HSD) solution (Lot No.: NC 54845) (AB Pharmacia, Uppsala, Sweden). Blood samples were withdrawn prior to and 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72, 96 and 168 h after the HSD infusion.

Adult, female New Zealand white rabbits (Elkhorn Rabbitry, Watsonville, CA) initially weighing 2.5 to 3.5 kg, were randomly assigned to either the hemorrhage (n=4) or control (n=4) group. Rabbit studies followed a similar protocol as the pigs except they were bled only 8 ml/kg body weight over a 15 min period to mimic a moderate hemorrhage since rabbits do not tolerate hemorrhage as well as pigs.

In a separate experiment, control New Zealand white rabbits were infused daily with HSD for 14 d at the maximum tolerated dose (MTD) of 16ml/kg. Blood samples were taken before infusion and on days 1, 3, 7, and 14 prior to subsequent infusions.

In vitro studies were also performed by mixing normal pig serum with varying concentrations of HSD to mimic the highest concentrations observed following daily infusion of HSD to experimental amimals at the MTD.

Human Studies

Plasma samples were obtained from 13 trauma patients infused with HSD (250ml) as part of the University of California Davis Medical Center Life Flight program. This study was approved by the Human Subjects Review Committee at the University of California at Davis. The patients enrolled in the present study were randomly selected from the larger test population.

The patients were transported to the hospital by a Life Flight helicopter system, and all had significant

hypotension upon entry into the study (systolic blood pressure <90). The HSD solution was administered intravenously, usually via percutaneously inserted catheters, over a period of 2-5 min en route to the hospital. All other aspects of the patients' care were those normally used. Additional fluid (Lactated Ringer's) was administered as clinically indicated. The time of administration of the HSD was noted and all additional fluid volumes given were recorded.

Upon arrival in the emergency room, a venous blood sample was taken for the measurement of hematocrit and plasma total protein, glucose, total carbohydrate, sodium and potassium levels.

Biochemical Measurements

Total carbohydrate concentrations in plasma were determined by the anthrone reaction following precipitation of both fluids with 10% trichloroacetic acid (TCA) (16,17). Plasma glucose was determined by an automated glucose-hexokinase enzymatic method performed by the Analytical Chemistry Branch, Letterman Army Institute of Research. Plasma dextran concentrations were then estimated by subtracting the glucose concentrations from the concentrations of total carbohydrate.

Serum or plasma protein was determined by 4 separate assays.

a) The standard biuret assay was performed by the Analytical Chemistry Branch using a Cobas Fara II centrifugal fast analyzer (Roche Analytical Instruments, Belleville, NJ). In this procedure, samples are centrifuged and the resultant clear supernatant read for protein.

b) The second method utilized a commercial dye-binding assay (BioRad Laboratories, Richmond, CA).

c) A modified Lowry assay utilizing a bincinchoninic acid color reagent rather than Folin reagent (Pierce, Rockford, IL) was also used.

d) Refractometry was included as a non-specific assay for total serum solute concentrations due to its simplicity and our main interest in following trends following dextran infusion.

Refractometry utilized a National refractometer (National Instrument Co, Inc, Baltimore, MD) and all readings were performed by the same investigator.

Statistical Analysis

In all studies, individual protein measurements from all assays performed were analyzed by analysis of variance with $p<0.05$ considered statistically significant. Where appropriate, repeated measures ANOVA was performed. Newman-Keuls multiple range test was used for post-ANOVA comparisons.

Results

When the biuret assay was employed, adding dextran as HSD, in vitro, to normal pig serum resulted in a 13% increase in protein concentration readings at dextran concentrations of 1200 mg/dl (Table 1). This increased to 65% higher readings at dextran concentrations of 2400 mg/dl. Refractometry showed a progressive increase in protein concentration as dextran concentrations increased from 600 to 3600 mg/dl. In contrast, no interference was observed when the dye-binding or modified Lowry assay was employed, even at dextran concentrations as high as 3.6 g/dl. (Table 1).

The next series of experiments examined possible dextran interference with serum protein assays following infusion of a "therapeutic" dose (4 ml/kg) of HSD to both euvolemic (control) and hemorrhaged swine. The hemorrhaged group reflects the clinical scenario for HSD use. As shown in Fig 1A, infusion of HSD to euvolemic pigs resulted in no increase in protein concentration that would have suggested interference from dextran. This observation was consistent among all protein assays employed although protein readings, in general, tended to be slightly higher with the modified Lowry assay, than the other 3 methods. Interference in serum protein readings following HSD infusion in hemorrhaged pigs was also not observed (Fig 1B), although hemorrhaged pigs had 28% higher peak serum dextran concentrations than their euvolemic counterparts (545 ± 32 vs. 426 ± 30 mg/dl). In hemorrhaged pigs, all 4 assays followed the decrease in serum protein with hemorrhage and the subsequent hemodilution induced by HSD (Fig 1B). Similar observations were observed in euvolemic and hemorrhaged rabbits (data not shown).

In a separate experiment, serum protein concentrations were determined in rabbits infused daily for 14 days with HSD at the maximum tolerated dose of 16 ml/kg (i.e., 4 times the proposed single therapeutic dose of 4 ml/kg). As shown in Fig 2, an increase in serum protein concentrations was observed with the biuret assays over the 14 d period, suggesting interference from dextran. Although protein concentrations were higher at day 1 than day 0 when assayed by the dye-binding or modified Lowry methods, no such increase was observed at subsequent days. No change at any day was observed by refractomety (Fig 2).

To further evaluate protein changes induced by dextran, plasma protein concentrations were determined in 13 trauma patients who received a 250 ml bolus of HSD (3.6 ± 0.2 ml/kg). The time from initiation of the dose of HSD to collection of the blood samples was 26.7 ± 4.5 min. Plasma dextran concentrations ranged from 115 to 458 mg/dl in these patients. As indicated in Fig 3, protein concentrations agreed well among all 4 assays employed and no dextran induced positive error in plasma protein measurements could be shown in these patients.

DISCUSSION

Numerous reports in the literature have cited interference from dextran in the estimation of serum protein concentrations (10-15). Since the biuret assay has been adapted to automated procedures and is commonly used in clinical chemistry labs, one approach to reduce interference is modifying the biuret color reagent (13-15). However, the universal practicality of this approach has yet to be adequately investigated. In the present study we monitored serum or plasma protein concentrations using standard methods, following infusion of dextran as HSD in experimental animals and trauma victims.

The studies in experimental animals were designed to mimic the hypovolemic states for which HSD is proposed. Therefore, it was of interest to determine if interference of serum protein measurements could be detected or if one assay method may be more reliable in the presence of dextran. Following the infusion of a single dose of HSD at the proposed therapeutic dose of 4 ml/kg, peak serum dextran concentrations were nearly 30% higher in hemorrhaged animals than their euvolemic controls. Nevertheless, no indication of dextran interference with serum protein determinations could be

detected with any of the 4 assays in either group, even when serum dextran concentrations exceeded 600 mg/dl.

In general, the dextran concentrations achieved in these studies were low compared to earlier studies using previous clinical dextran preparations where 800 to 1400 mg/dl dextran concentrations were reported (cf 12). Others even reported that 2000 mg/dl dextran concentrations could be achieved if multiple infusions were administered (cf 13). These differences in serum dextran concentrations between these and the present study reflect the reduction in dose an total dextran load infused, associated with the therapeutic use of HSD [5].

In the present study we took two different approaches to investigate the effects of high serum dextran concentrations on serum protein determinations. The first experiment utilized serum samples from rabbits employed in a subacute HSD toxicity study (18). Rabbits were infused daily for 14 days with the maximum tolerated dose of HSD (16 ml/kg). Serum dextran concentrations exceeded 1000 mg/dl by day 3 and were as high as 2500 mg/dl on day 14 in these animals. Serum protein measurements increased with the biuret method when dextran concentrations exceeded 1000 mg/dl, but no dose-response effect could be detected. The other assays employed did not indicate any specific evidence of dextran-induced interference, despite these high serum concentrations. Since dextran induces fluid and protein shifts (6,8), the potential for these actions made it difficult to predict the particular changes which could be expected over the experimental period following daily HSD infusions. Therefore, a separate experiment examined the direct in vitro effects of dextran and/or salt concentrations on serum protein. It was observed that when dextran concentrations increased from 600 to 3600 mg/dl, refractometric measurement of serum solute concentrations showed a dose-response increase, consistent with the early report of Aronsson, et al (10). Also, even though our biuret reagent contained the standard 9 g/l of Na-K tartrate, use of the centrifugal analyzer appeared to greatly diminish the possibility for dextran interference, as has been previously shown (10).

Finally, for practical application of protein concentration measurements to humans infused with dextran, we assayed plasma from trauma patients administered a standard 250 ml dose of HSD. Plasma protein concentrations were consistent among the 4 assays used, at least in this situation where plasma

dextran concentrations ranged from 115 to 458 mg/dl. In this trial blood samples had been drawn about 30 min following HSD administration. Therefore, considering a half-life for serum dextran of about 10 to 12 hr (5), it would appear that significantly higher dextran concentrations would not be observed if the samples had been drawn sooner.

In conclusion, the data from the present study indicate that at dextran concentrations observed or expected following a single dose of HSD for the treatment of hypovolemia, accurate measurement of serum protein concentrations will not be affected. In addition, any of the 4 assays are suitable for these measurements and if an automated centrifugal analyzer is used for biuret assays, modification of the color reagent is probably not necessary.

References

1. Velasco IT, Pontierri V, Rocha e Silva M. Hyperosmotic NaCl and severe hemorrhagic shock. Am J Physiol 1980; 239: H664-H673.

2. Maningas PA, DeGuzman LR, Tillman FJ, Hinson CS, Priegnitz KJ, Volk KA, Bellamy RF. Small-volume infusion of 7.5% NaCl in 6% Dextran 70 for the treatment of severe hemorrhagic shock in swine. Ann Emerg Med 1986; 15(10): 1131-1137.

3. Hands R, Holcroft JW, Perron PR, Kramer GC. Comparison of peripheral and central infusion of 7.5% NaCl/6% dextran 70. Surgery 1988; 103:684-689.

4. Chudnofsky CR, Dronen SC, Syerud SA, Zink BJ, Hedges JR. Intravenous fluid therapy in the prehospital management of hemorrhagic shock: Improved outcome with hypertonic saline/6% Dextran-70 in a swine model. Am J Emerg Med 1989; 7:357-363.

5. Dubick MA, Summary JJ, Ryan BA, Wade CE. Dextran concentrations in plasma and urine following administration of 6% Dextran-70/7.5% NaCl to hemorrhaged and euvolemic conscious swine. Circ Shock 1989; 29:301-310.

6. Wade CE, Hannon JP, Bossone CA, Hunt MM, Loveday JA, Coppes R, Gildengorin VL. Resuscitation of conscious pigs following hemorrhage: Comparative efficacy of small volume resuscitation. Circ Shock 1989; 29:193-204.

7. Halvorsen L, Ashley KD, Hands RD, Nakayama S, Smith GJ, Holcroft JW, Kramer GC. Measurement of rapid plasma volume expansion after infusion of hypertonic saline using changes in plasma protein concentrations. Circ Shock 1989; 27:319.

8. Kramer GC, Gunther RA, Nerlich ML, Zweifach SS, Demling RH. Effect of Dextran-70 on increased microvascular fluid and protein flux after thermal injury. Circ Shock 1982; 9:529-541.

9. Summary JJ, Zaucha GM, Dubick MA, Parker RJ, Wade CE, Korte Jr DW. Serum immunoglobulin, complement and protein responses to 7.5% NaCl/6% Dextran-70 (HSD) in euvolemic dogs. FASEB J 1990; 4:A1085.

10. Aronsson T, Arturson G, Wallenius G. Determinations of serum protein in the presence of dextran. Scand J Clin Lab Invest 1966; 18:458-460.

11. Crowley LV. Interference with certain chemical analyses caused by dextran. Am J Clin Pathol 1969; 39:425-426.

12. Reichel J, Hassan A, Teubel B, Gockeritz D. In vitro interference due to dextran when determining protein. Pharmazie 1989; 44: 725-726.

13. Moore JJ, Sax SM. Elimination of dextran interference in serum protein determinations. Clin Chem 1972; 18:393-394.

14. Flack CP, Woollen JW. Prevention of interference by dextran with biuret-type assay of serum proteins. Clin Chem 1984; 30:559-561.

15. Hadjivassiliou AG, Benou NS, Diskakis EJ. Total serum protein quantified without interference from dextran. Clin Chem 1988; 34:2388.

16. Scott TA Jr, Melvin EH. Determination of dextran with anthrone. Anal Chem 1953; 25:1656-1661.

17. Roe JH. The determination of dextran in blood and urine with anthrone reagent. J Biol Chem 1954; 208:889-896.

18. Zaucha GM, Frost DF, Omaye ST, McKinney LA, Pearce MJ, Korte, DW Jr. Fourteen day subacute intravenous toxicity study of hypertonic saline/Dextran-70 and its constituents in New Zealand White rabbits. Letterman Army Institute of Research Institute Report #405, 1989.

Table I

Effects of Dextran and/or Salt, in vitro, on Swine Serum Protein Concentrations[1]

		Protein Assay			
Dextran (mg/dl)	NaCl(%)	Biuret	Dye-Binding	Mod-Lowry	Refractometry
150	0.19	5.5	6.0	6.6	5.6
0	0.19	5.5	5.9	7.1	5.0
300	0.38	5.6	5.9	6.7	5.8
0	0.38	5.5	5.5	6.9	5.0
600	0.75	5.5	5.5	6.9	6.0
0	0.75	5.3	5.7	6.8	5.4
1200	1.25	6.2	6.0	7.7	7.1
0	1.25	5.0	6.3	7.3	5.0
2400	2.5	8.9	5.5	7.0	9.0
0	2.5	5.0	5.6	7.0	7.8
3600	3.75	10.3	5.8	7.6	10.7
0	3.75	5.1	5.9	7.4	10.4
0	0	5.4	5.7	7.4	5.4

[1]Data represent average of 2 to 3 readings except biuret assay is a single reading.

Legend to Figures

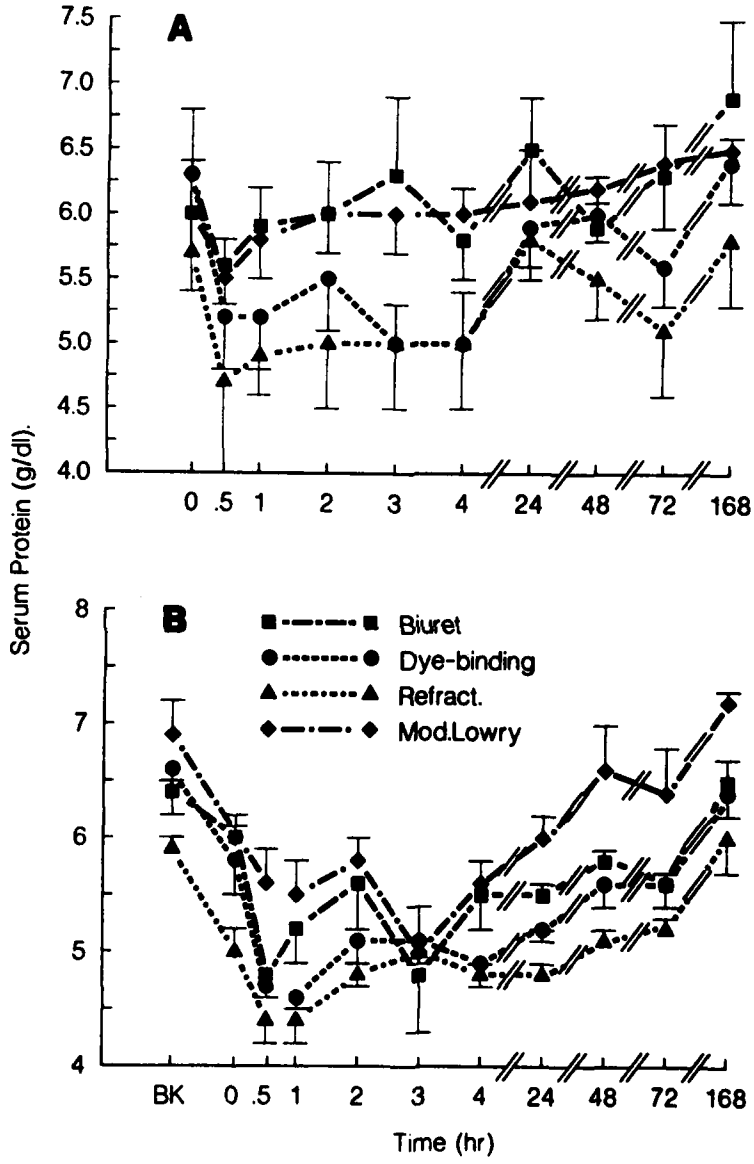


Fig 1. Serum protein concentrations in A) Euvolemic and B) Hemorrhaged pigs infused with 4ml/kg HSD. BK: Background or baseline reading. Data represent mean ± S.E. of 4 animals/group.

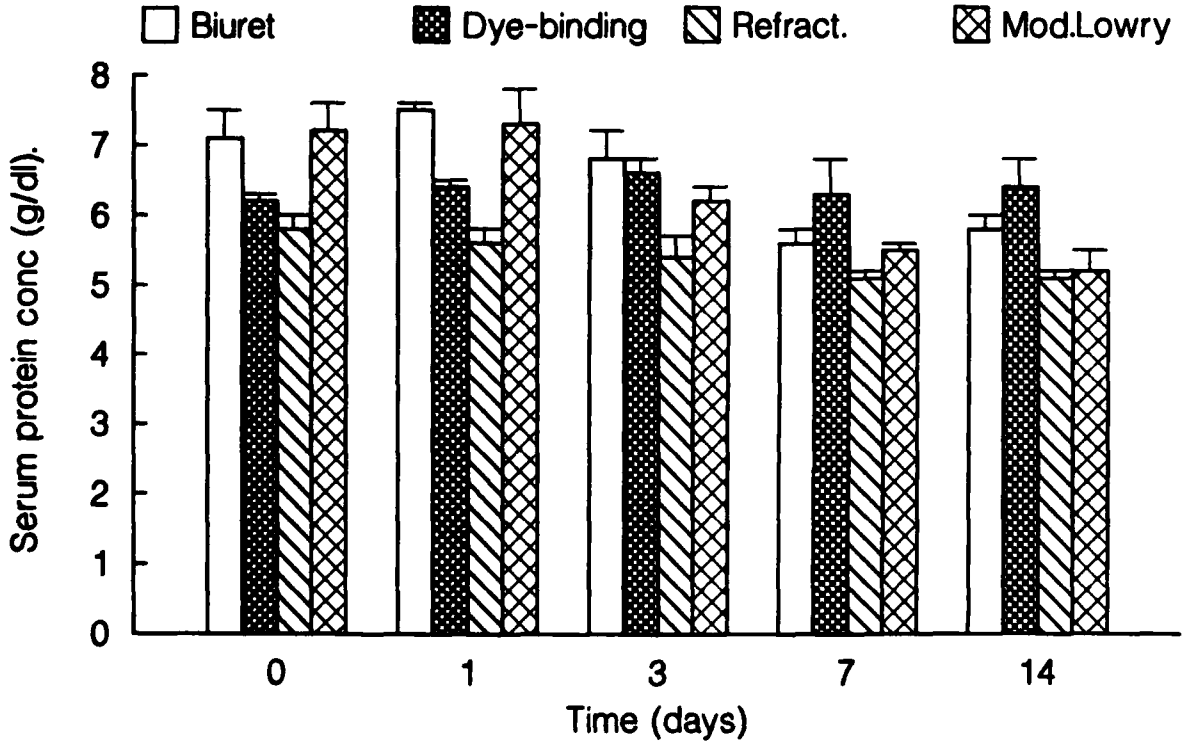


Fig 2. **Serum protein concentrations in euvolemic rabbits infused daily with 16ml/kg HSD for 14 days.** Data represent mean ± S.E. of 4 animals.

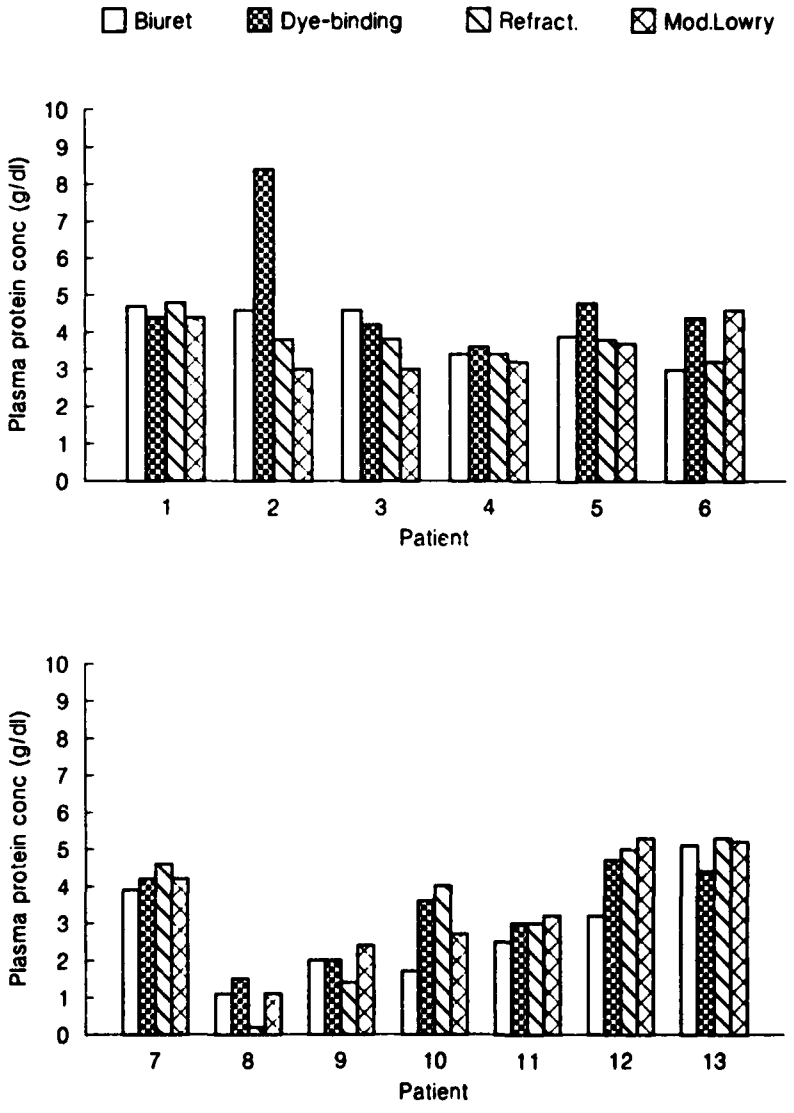


Fig 3. Plasma protein concentrations in 13 trauma patients administered a 250ml dose of HSD. Data represent single determination by each assay.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
& Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

DASG-AAFJML
Army/Air Force Joint Medical Library
Offices of the Surgeons General
5109 Leesburg Pike, Room 670
Falls Church, VA 22041-3258

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University of
Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD-UWZ-L
Walter Reed Army Institute of Research
Washington, DC. 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering Research
and Development Laboratory
ATTN: SGRD-UBG-M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Research Institute of
Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute of
Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research
Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

USAF School of Aerospace Medicine
Document Section
USAFSAM/TSKD
Brooks Air Force Base, TX 78235-5301

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000

Commander
Naval Medical Command-02
Department of the Navy
Washington, DC 20372-5120